Implicating Dysbiosis of the Gut Fungal Microbiome in Uveitis, an Inflammatory Disease of the Eye

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PURPOSE. In this study, the gut fungal microbiome of uveitis (UVT) patients was generated and compared with healthy controls (HC) to identify dysbiosis in UVT patients and ascertain the role of gut fungal microbiome in disease pathology.

METHODS. In the present study, gut fungal microbiomes were analyzed in the fecal samples of HC (n = 24) and UVT patients (n = 14) using high-throughput Illumina sequencing of ITS2 region of the fungal ribosomal RNA. QIIME and R software were used for data analysis.

RESULTS. The gut fungal richness and diversity were significantly decreased in UVT patients compared with HC. Our analyses showed enrichment of several pathogenic fungi including Malassezia restricta, Candida albicans, Candida glabrata, and Aspergillus gracilis in UVT patients. Heatmap and discriminatory OTUs further confirmed the disparities between UVT and HC microbiomes.

CONCLUSIONS. This is the first study demonstrating dysbiosis in the gut fungal communities of UVT patients indicating the importance of fungal microbiome in the disease pathology. These initial findings might warrant further investigation into the fungal microbiome, especially interactions between fungal and bacterial that then might give further insight into how probiotics or fecal transplants might benefit.

Keywords: uveitis, gut fungal microbiome, dysbiosis, illumina miseq

Uveitis (UVT) is a sight-threatening inflammatory disease of the uveal tract (iris, ciliary body, and choroid) of the eye. It is the fifth most common cause of vision loss in the developed world and is responsible for 10% of the legal blindness in the United States. The population prevalence of UVT in India was estimated to be approximately 750 per 100,000, which is higher than in developed countries. The treatment for UVT is based on nonspecific immune-suppression by using topical and oral corticosteroids, antimetabolites, and alkylating agents. However, due to the adverse side effects, there is a need to develop alternative therapies that will help in improving the treatment of UVT.

A number of studies have demonstrated altered bacterial communities (dysbiosis) in the guts of diseased individuals compared to healthy individuals. A few of these studies in humans have also shown association of dysbiosis in the gut bacterial microbiome with UVT. Studies in mice models also suggested a role for the gut microbiome in the pathophysiology of UVT and indicated that altering the bacterial composition would attenuate the disease. The most probable mechanism by which gut microbiome influences disease pathology is through the interaction with host immune system.

Studies showing association of gut fungal microbiome with diseases are less compared to bacterial microorganisms, which may be attributed to the several challenges that are faced in characterizing fungal communities right from extraction of genomic DNA to molecular identification, the latter mainly due to the presence of high sequence length variability in fungal sequences (ITS1 and ITS2) and due to the incomplete availability of fungal reference sequence databases. Despite these challenges, dysbiosis in fungal microbiome has been implicated in several intestinal diseases/disorders like colorectal adenomas, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS), and also in few other diseases like myalgic encephalomyelitis/chronic fatigue syndrome, diabetes, and cirrhosis. Few studies have demonstrated an increased immune sensitivity to fungal antigens in UVT implying that these fungal antigens may be an important risk factor of idiopathic UVT, especially for those associated with spondyloarthritis and multiple sclerosis. In addition, Zarate-Bladés et al., who had characterized only the bacterial populations, failed to identify the antigenic mimic that triggers UVT in the mouse model of spontaneous UVT thus implying that other microbial communities including fungi may help to identify the trigger for UVT. Recently we showed association of gut fungal microbiome with bacterial keratitis. Hence, it is worthwhile to investigate fungal microbiome in UVT patients. The present study aims to characterize the gut fungal microbiomes of healthy controls (HC) and UVT patients from a south Indian cohort to identify whether gut fungal populations are altered in UVT patients and whether dysbiosis in gut fungal communities is associated with UVT that may help to develop new treatment regimen for treating UVT.
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**Materials and Methods**

**Recruitment of Subjects**

Patients with idiopathic \((n = 12)\), auto-immune UVT, which included patients with Vogt–Koyanagi–Harada disease \((n = 2)\) were recruited at the L. V. Prasad Eye Institute, Hyderabad, India. The UVT patients included 12 women and 2 men who were between 22 and 63 years of age, with mean ± SD age of 43.64 ± 14.37 years (Supplementary Table S1). HC individuals \((n = 24)\) who were age (22–81 years with mean ± SD age of 45.92 ± 16.91; \(P = 0.676\)), ethnicity \((P = 1.000)\), and diet \((P = 1.000)\) matched with the UVT group were only included. The HC and UVT patients were from Telangana state in India. The sample size was calculated using the population-proportion method with confidence level 95%. The population prevalence of UVT in India was estimated to be approximately 730 per 100,000.\(^1\) Exclusion criteria for all HC and UVT subjects was as follows: participants who had taken probiotics, prebiotics, or antibiotics 3 months prior to sample collection; or had undergone gastrointestinal (GI) tract surgery; individuals with a systemic disease (like diabetes, tuberculosis, HIV, sarcoïdosis, hypertension, obesity, IBD, prolonged constipation or diarrhea, arthritis, and any other systemic infection or inflammation); patients with diabetic retinopathy (proliferative or nonproliferative), wet age-related macular degeneration, myopic degeneration with active subfoveal choroidal neovascularization, ocular malignancy in either eye including choroidal melanoma, or any form of malignancy were not included in the study cohort. Informed consent was taken from all the study subjects prior to sample collection. The study protocols were approved by the Institutional Review Board of L. V. Prasad Eye Institute, Hyderabad (Ethics Ref. No. LEC 06-14-060) and adhered to the tenets of the Declaration of Helsinki.

**Fecal Sample Collection and DNA Extraction**

Stool samples were collected in a sterile container (HiMedia, India) by the subjects and were frozen at –80°C until further processing. Homogenized fecal sample (300 mg) was used for extraction of genomic DNA using QIAamp DNA stool minikit (Qiagen, Hilden, North Rhine-Westphalia, Germany) according to the manufacturer’s instructions. DNA extractions were carried out in triplicate. PCR amplification and sequencing were done with the DNA sample obtained by pooling equal volume of DNA from each replicate. Genomic DNA was checked for quality on a 0.8% agarose gel and quantified using Qubit 2.0 fluorometer (Life Technologies, India).

**Amplification by PCR, Illumina Library Preparation, and Amplicon Sequencing**

ITS2 region of the fungal ribosomal RNA was amplified using the primers ITS3 \((5’-GCACTGAAGAAGCCAGC-3’)\) and ITS4 \((5’-TCCCCCTATTGATATGCG-3’)\).\(^20\) Fungal microbiomes were generated from the ITS2 amplicons by using the standard Illumina protocol described by Dehingia et al.\(^21\) Sequencing of the libraries was performed using 2 × 250 bp chemistry on an Illumina MiSeq with paired-end protocol at the Xcelris Genomics Pvt. Ltd. (Ahmedabad, India).

**Taxonomic Classification of Sequenced Reads**

Demultiplexed paired-end reads (Fastq) of each sample were combined into contigs using FLASH software run with default parameters.\(^22\) Prinseq-lite\(^23\) and Usearch\(^6\) were used for quality filtering (reads with mean Phred score of <25 were removed) and chimera removal respectively to obtain high-quality (HQ) reads, which were used for operational taxonomic unit (OTU) picking as implemented in QIIME. Sequences were clustered with 97% similarity using UCLUST run with default parameters.\(^25\) UNITE OTUs (ITSS Alpha release) clustered at 97% identity was used as the reference OTU database. Taxonomic assignment for the OTUs, which matched the reference OTU database (reference-OTUs), was obtained as provided in the database, whereas Wang Classifier\(^26\) (with bootstrap threshold of 80%) implemented in MOTHUR (version v.1.29.2) was used for assigning taxonomy for de novo clustered OTUs (denovo-OTUs). Sparse OTUs (representing <0.001% of the total HQ reads) were excluded from the analysis.

**Diversity Analyses of Fungal Microbiomes**

Rarefaction curves were plotted using R-Vegan 2.4-2package.\(^27\) \(\alpha\) diversity indices (Shannon diversity, Simpson index, observed number of OTUs, and Chao1) were obtained to ascertain the degree of variation.

**Identification of Differentially Abundant Taxonomic Groups**

Wilcoxon signed rank test was performed to identify the taxonomic groups (at phylum, genus, and OTU level) in the fungal microbiome, which were differentially abundant in HC and UVT samples (Benjamini Hochberg [BH] corrected \(P < 0.05\)). Nonmetric multidimensional scaling (NMDS) plots of microbiome samples were generated (using Bray-Curtis dissimilarity) based on OTUs.

**Correlation Network Between Bacterial and Fungal Genera**

Bacteria-fungi interaction in the microbiomes was visualized by generating two interactive networks specific to the HC and UVT microbiomes separately. Pearson correlation coefficient \((r)\) was used to obtain the pair-wise correlations between abundances of the genera. The networks were visualized and analyzed using Cytoscape\(^28\) and CoNet.\(^29\) The bacterial microbiome data of nine UVT patients and 13 HC was obtained from our earlier study.\(^3\)

**Results**

The data reported in the present study are part of a larger study involving the comparison of variations in the gut bacterial and fungal microbiomes in HC and individuals with bacterial keratitis, fungal keratitis, or UVT. For instance, using the same set of HC, we compared the bacterial and fungal microbiomes of individuals with bacterial and/or fungal keratitis or UVT with the microbiomes of HC matched for age, gender, ethnicity, and region of origin.\(^4\) Such an approach is advantageous since by carefully designing the experiments the same set of control individuals could be recruited for the three different ocular diseases. In this paper, the demographic and the fungal microbiome data of 24 HC was derived from the 31 individuals analyzed by us earlier\(^4\) and was also common to 21 HC,\(^19\) which were published earlier. The demographic data of 13 of the 14 UVT patients is also common to the UVT patients in Kalyana Chakravarthy et al.\(^4\)

**Rarefaction Analysis and \(\alpha\) Diversity Indices of the Gut Fungal Microbiomes of HC and UVT Patients**

A total of 38 fungal microbiomes were generated from the fecal samples of 24 HC and 14 UVT patients, which yielded 34.3
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Analysis of the Fungal Communities in the Gut Fungal Microbiomes of Healthy Individuals and UVT Patients

The phyla Ascomycota (32.03% mean abundance) and Basidiomycota (mean abundance, 24.29%) were the dominant phyla in HC and UVT patients (Fig. 1C; Supplementary Table S4). The abundance of these two phyla between HC and UVT microbiomes was not statistically significant. However, significant variation (BH corrected \( P < 0.05 \)) was observed in the low abundant phyla Glomeromycota, Chytridiomycota, and Zygomycota (mean abundance <1%). (Fig. 1C; Supplementary Table S4). It was also noted that a substantial amount of reads (mean abundance, 35.43%) were unclassified at the phylum level indicating the presence of several unknown fungal taxonomic groups in the guts of Indian subjects and also reiterates the limitations in the existing fungal sequence databases.

However, detailed analysis at the OTU level (median abundance >0.001) revealed significant (BH corrected \( P < 0.05 \)) differential abundance in the gut microbiomes of HC and UVT patients with 52 OTUs diminished in UVT patients and 21 OTUs enriched in UVT patients (Table). Based on taxonomic annotation of the OTUs (Supplementary Table S5), 67 discriminatory genera could be identified in the gut microbiomes of HC and UVT patients. Out of these, 14 genera were common to both HC and UVT microbiomes, 45 genera were unique to HC, and 8 genera were unique to UVT. Further, the HC and UVT fungal microbiomes could be discriminated significantly with respect to 17 genera (median abundance >0.001%) (Fig. 2). However, the two-dimensional heatmap of the 17 discriminating fungal genera, based on hierarchical clustering of rank normalized abundances (scaled between 0 and 1), did not yield a clear distinct discrimination between HC and UVT (Fig. 3A). The 24 HC gut microbiomes formed 2 clusters (15 and 9 microbiomes), and the 14 UVT microbiomes formed a single cluster (Fig. 3A). NMDS analysis also indicated that the HC formed two different clusters, and both clusters are distinct from UVT microbiomes (Fig. 3B). Further, it was possible to discriminate the HC microbiomes in two clusters affiliated to Basidiomycota and the other to Ascomycota, respectively (Fig. 3C). Both these clusters were significantly distinct from the UVT microbiomes (Fig. 3B). Further, when NMDS plot of HC and UVT was drawn, it yielded two clusters (green corresponding to HC and red to UVT). The \( P \) value

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Lineage</th>
<th>Number of OTUs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTUs diminished in UVT compared to HC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Acroconidiella sp. HSAU074066</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Calyptella capula</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Candida albicans</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Embellista sp. DAR 74619</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Fungal sp. mh15858</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Fusarium solani</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Gibberella sp. CBS 119214</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Lastidiopodina pseudotuberculata</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Mortierella alpina</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Mortierellales sp. QLF84</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Mycocentrospora sp. UFMGCB 2526</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Pichia exigua</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Pleosporales sp. REF111</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Preussia tetramer</td>
<td>1</td>
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<tr>
<td>15</td>
<td>Rhizopus oryzae</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>Rhizoscyphus ericae</td>
<td>1</td>
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<tr>
<td>17</td>
<td>Stagonosporopsis curvatae</td>
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<tr>
<td>18</td>
<td>Uncultured Neotia nidus avis</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>Uncultured fungus</td>
<td>3</td>
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<tr>
<td>20</td>
<td>Uncultured Guebomyces</td>
<td>1</td>
</tr>
<tr>
<td>21</td>
<td>Uncultured Mortierella</td>
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</tr>
<tr>
<td>22</td>
<td>Uncultured rhizosphere ascomycete</td>
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</tr>
<tr>
<td>23</td>
<td>Uncultured root associated fungus</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>Uncultured zygomyzete</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>Unclassified</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>

| OTUs enriched in UVT samples compared to HC | | |
| 1 | Aspergillus gracios | 1 |
| 2 | C. albicans | 3 |
| 3 | Candida glabrata | 2 |
| 4 | Issatchenkia sp. NUC 7766 | 1 |
| 5 | Malassezia globosa | 1 |
| 6 | Malassezia restricta | 1 |
| 7 | Saccharomyces cerevisiae | 1 |
| 8 | Uncultured Basidiomycota | 1 |
| 9 | Uncultured fungus | 1 |
| 10 | Uncultured soil fungus | 1 |
| 11 | Unclassified | 8 |
| Total | | 21 |

* OTUs having a median abundance of >0.001% in at least one group (HC or UVT) are listed. All the OTUs listed above exhibited significant differential abundance (BH corrected \( P < 0.05 \)) in the gut microbiomes of HC and UVT patients.

Interactions of Bacterial and Fungal Genera in the Gut Microbiomes of HC and UVT Patients

The HC interaction network consisted of a central major network and several small disjointed networks with both bacteria and fungi interacting (Fig. 4A). This central major network was made up of seven bacterial hubs represented by Prevotella, Veillonella, Roseburia, Oscillatori, Slackia, Collinsella, and Dialister interacting with more than five other taxa. Three of these bacterial hubs Slackia, Collinsella, and Dialister interacted with both bacteria and fungi. Gibberella, Termitomyces, Volvariella, Echinoderm, Gymnopus, and Cochliobolus constituted the six fungal hubs in the central
major network, and only the hubs of *Gibberella* and *Cochliobolus* interacted with both bacteria and fungi, whereas the others interacted exclusively with other fungi. In addition to the central network in HC, 10 other disjointed networks were observed made up of two to eight taxa and consisted of bacteria, fungi, or a mix of bacteria and fungi (Fig. 4A). In the HC network, most of the bacterial taxa exhibited negative interactions, whereas the fungi exhibited positive interactions.

In the UVT interaction network also a central major network was observed with nine bacterial and five fungal
hub genera (Fig. 4B). *Megasphaera* and *Leptosphaerulina* were the biggest bacterial and fungal hubs, respectively. Majority of the taxa exhibited negative interactions. The eight disjointed networks were small, and the number of taxa ranged from 2 to 12. It is interesting that in the largest of the disjointed networks, all the bacteria and fungi were hubs exhibiting interaction with more than five other taxa, and they all exhibited only positive interaction.

**DISCUSSION**

The most comprehensive study on the human gut fungal microbiome was part of the Human Microbiome Project cohort where 317 stool samples were analyzed by ITS2 sequencing. The study outlined that fecal fungal diversity was significantly lower in comparison to bacterial diversity and yeasts were the most predominant genera represented by *Saccharomyces*. 

**FIGURE 2.** Fungal genera exhibiting significant differential abundance (BH corrected $P < 0.05$) in the gut microbiomes of UVT patients compared to HC. Genera having a median abundance of $>0.001\%$ in at least one group of samples (HC or UVT) have been depicted. Median abundances (horizontal line) and interquartile ranges have been indicated in the plots.
cerevisiae, Malassezia restricta, and Candida albicans OTUs in >80% of samples. Further, the inter- and intravolunteer variability in fungal communities was very high. Despite the variability, Nash et al. proposed that the presence of several fungal species common to majority of fecal samples was indicative of the presence of a core gut fungal microbiome. However, in difference to Nash et al., it has been proposed that there is no “normobiosis” or core gut fungal microbiome in healthy individuals. We also could not identify a core fungal microbiome in the HC individuals. The reasons for the lack of a core gut fungal microbiome could be attributed to the observation that Ascomycota and Basidiomycota, which are the dominant phyla, were mostly inversely correlated, thus creating two groups of HC in which presence of either Ascomycota or Basidiomycota were mutually exclusive. In confirmation, we observed that the 24 HC gut fungal microbiomes formed three clusters with two clusters representing HC and a single cluster representing UVT. (C) Further NMDS analysis of only HC indicated that HC formed two clusters with dominance of Ascomycota in one and dominance of Basidiomycota in the other cluster.

**FIGURE 3.** Heatmap analysis, NMDS plots indicating gut fungal microbiome dysbiosis in UVT patients. (A) Two dimensional heatmap depicting rank normalized abundances (scaled between 0 and 1) of the 17 fungal genera (median abundance >0.001%), which were differentially abundant in the gut microbiomes of HC compared to UVT patients. The discriminating genera and the samples (HC and UVT) are arranged along the two dimensions (axes) based on hierarchical clustering. (B) β diversity analysis using the NMDS plots using Bray-Curtis dissimilarity of OTUs indicated that HC and UVT microbiomes formed three clusters with two clusters representing HC and a single cluster representing UVT. (C) Further NMDS analysis of only HC indicated that HC formed two clusters with dominance of Ascomycota in one and dominance of Basidiomycota in the other cluster.
most common genera present in more than 42% of samples. Subsequent studies also added *Penicillium* and *Wallemia*, known human symbionts *Cryptococcus, Malassezia*, and *Trichosporon* spp., and food-associated fungi *Debaryomyces benseni*, and *Penicillium roqueforti*. In the current study, we detected 137 genera in HC fungal microbiomes out of which 58 genera were present in more than 40% of the fecal samples (Supplementary Table S5). Overall, in compliance with earlier studies, we detected a huge number of fungal genera in the gut mycobiota including known human symbionts (*Cryptococcus, Malassezia*, and *Trichosporon* spp.), food-associated fungi (*P. roqueforti*), edible mushrooms (*Termitomyces, Volvariella, Agaricus, Chlorophyllum, Coprinopsis*), plant pathogens (*Embellisia, Mycocentrospora*), and other organisms, which may not have a specific role in the mammalian GI tract. *Candida, Fusarium*, and *Mortierella* were present in all the samples.

The primary objective of this study was to ascertain whether dysbiosis in the gut fungal microbiome was associated with UVT patients. In this study, we compared the gut fungal microbiome of HC versus UVT and specifically chose only UVT individuals either with auto-immune UVT or idiopathic UVT. Earlier studies had indicated dysbiosis in the gut bacterial microbiome of rats and mice with auto-immune UVT. Our own study also confirmed gut bacterial microbiome dysbiosis in human beings with auto-immune and idiopathic UVT. Two papers indicated that HLA B27 affects the microbiome and in turn predisposes to UVT. Systemic inflammatory diseases like asthma, diabetes, rheumatoid arthritis, ulcerative colitis, Crohn’s disease, etc., have also been implicated in bacterial
gut microbiome dysbiosis, and therefore such individuals were not recruited. Comparison of the four diversity indices viz. Shannon diversity index, Simpson index (evenness), number of observed OTUs, and Chao1 index (richness) indicated that both the number of species and diversity was higher in HC compared to UVT patients (Fig. 1B). Similar decrease in diversity and abundance was observed in patients with other diseases such as in obese individuals and in patients with asthma, cirrhosis, and autism.

At the phylum level, Ascomycota and Basidiomycota were the most dominant in the gut microbiomes of HC and UVT patients, and this observation is in congruence with Bhute et al. and our own studies on the gut fungal communities from an Indian population. Ascomycota and Basidiomycota were also dominant in healthy subjects and IBD patients. We also observed that a large proportion of reads (mean abundance, 35.30%) remained unclassified at the phylum level, indicating the limited availability of fungal sequence databases.

At the genera level, 17 genera (median abundance >0.001%) could discriminate the HC and UVT microbiomes, implying dysbiosis in the fungal microbiome of UVT patients. This discrimination was also observed following heatmap analysis and β diversity analysis using the NMDS plots. This is the first direct evidence implicating gut fungal microbiome dysbiosis in UVT, and it supports a few earlier investigations that provided clues of the involvement of the fungal microbiome in ocular diseases like UVT. The relevance of the observed dysbiosis in UVT patients would be all the more meaningful if functional attributes were associated with the genera that decreased or were enriched in UVT patients. Enrichment of five of the nine fungal genera in UVT patients was on expected lines since the enriched organisms like Aspergillus gracilis, Candida glabrata, Malassezia globosa, and M. restricta were opportunistic pathogens. Further, Issatchenkia sp. AUMC 7766 is an emerging pathogen. It was also observed that in HC individuals, 24 genera of yeasts...
were more in abundance, and these may be beneficial to the HC due to their anti-inflammatory or antipathogenic effects. *Embellisia* sp., *Mortierella alpina*, and *Mortierellales* sp. are probably anti-inflammatory as observed in *Embellisia eureka* and a few *Mortierella* species. One of them, *Preussia tetroniera*, has antimicrobial activity. In addition, we also observed that several genera recognized as plant/tree pathogens like *Acrocomidiella* sp., *Calypella capula*, *Gibberella* sp., *Lasiodiplodia pseudotomonomae*, *Mycocentrospora* sp., *Pleosporales* sp., *Rhizopus oryzae*, *Rhizoscyphus ericae*, and *Stagonosporopsis cucurbitacearum* and human pathogens like *Fusarium solani* and *Pichia exigua* were also diminished in UVT individuals. The reason for the observed increase in pathogens in HC is unexplainable but has been observed earlier. Decrease in fungal genera was also observed earlier in bacterial keratitis and IBD patients compared to controls.

Interactions between bacterial and fungal populations in a microbiome would be useful to predict outcomes from alterations in microbial community structures under a given physiological condition. The present study confirms earlier studies that indicated that interactions between bacteria and fungi in the gut microbiome of healthy individuals were either positive or negative, and in most cases the interaction appeared to be functionally relevant. For instance, in the present study, in HC microbiome *Dialister*, an SCFA producer, negatively interacted (i.e., inhibited) with pathogenic *Escherichia*, *Shigella*, *Sutterella*, *Coprinopsis*, and *Chlorophyllum*, a mushroom. *Dialister* also inhibited *Mortierella*, which has anti-inflammatory properties. It was also observed that *Gibberella*, a plant pathogen that was the biggest hub, also interacted negatively with many pathogenic fungi (like *Marasmius*, *Malassezia*, *Lasiodiplodia*, *Coprinopsis*, *Volvariella*, etc.) and bacteria (D. *Desulfovibrio*, *Streptococcus*, *Slackia*, and *Collinsella*). *Oscillospora*, the other major hub in HC network, is a probiotic organism that showed negative interaction with pathogens like *Slackia*, *Penicillum*, *Gemella*, and *Fusobacterium*. Another major hub was *Prevotella*, which interacted negatively with microbes that functionally could be characterized as pathogens (*Treponema*, *Rothia*, *Clostridium*, *Atopobium*), anti-inflammatory (*Bifidobacterium*, *Clostridium*, *Bacteroides*, *Butyrivirchimonas*, *Anaerostipes*), and probiotic (*Bifidobacterium*, *Veillonella*, and *Bacteroides*). It would appear that the majority of the interactions were directed toward controlling the dominance of pathogenic microbes in the HC gut microbiome and this is anticipated. In HC microbiome, we also observed that certain bacteria like *Oscillospora*, a probiotic organism, negatively interacted with butyrate, producing *Butyrivirchimonas* and *Anaerostipes*; *Dialister*, an SCFA producer, inhibited *Mortierella*, which has anti-inflammatory properties; and *Prevotella* interacted negatively with anti-inflammatory and probiotic microorganisms. Such interactions are not on anticipatory lines and are difficult to interpret.

*Megaspheara* (a pathogen) was the largest hub observed in the UVT microbiome interaction network, and the hub genus interacted with 12 other taxa. It negatively interacted with beneficial probiotic (*Butyrivirchimonas*, *Blautia*, and *Phasolocardobacterium*) and anti-inflammatory organisms (*Faeicacibacterium* and *Roseburia*). SCFA producers *Ruminococcus*, *Butyricimonas*, *Coprococcus*, *Roseburia*, *Lachnospira*, and *Faeicacibacterium* were also negatively inhibited by *Prevotella* (proinflammatory bacteria) and other hub bacteria or fungi. Such interactions that would suppress beneficial organisms would probably help to maintain the dysbiotic conditions. Unexpectedly, it was also observed that a few of the hub genera like *Megaspheara*, *Bifidobacterium*, and *Megamonas* negatively interacted with pathogens like *Sagenomella*, *Malassezia*, *Bifidobacterium*, *Shigella*, *Erwinia*, *Slackia*, *Atopobium*, *Lepthospaeraulina*, *Shigella*, *Pantoaea*, and *Pichia*, with *Saccharomycyes* and *Turicibacter*, which are probiotic in nature and with *Roseburia*, which is anti-inflammatory. The UVT microbiome interaction network also consisted of eight disjointed networks. One of the disjointed networks with eight bacterial and four fungal hubs appeared to be unique since every hub genera interacted positively with others in the network. This network included *Sbeurnella*, *Halomonas*, *Lasiodiplodia*, *Aggregatibacter*, *Clavispora* (which are pathogens), *Akkrermansia*, *Odoribacter*, *Acidaminococcus*, and *Kluyveromyces* (which are anti-inflammatory), *Pseudoadertomonas* (which is a probiotic and antibacterial), and two others, which included *Kazachstania* (an anaerobic bacterium) and *Anaerobrictob*, a gut microbe. Thus, in the gut microbiome of UVT patients, a few of the interactions were in support of the inflammatory condition like negative effects on beneficial organisms like those with probiotic and anti-inflammatory characteristics. But it was also observed that some of the hub genera also had negative interactions with pathogens and this is difficult to explain. Similar observations were made in the gut of patients with keratitis.

**Conclusions**

The α diversity indices viz. Shannon diversity index, Simpson index (evenness), number of observed OTUs, and Chao1 index (richness) were higher in HC compared to UVT patients, indicating that both the number of species and diversity were altered in the fungal gut microbiome of UVT patients. HC and UVT microbiomes could be discriminated at the OTU and genera level. In HC individuals, 24 genera of yeasts with anti-inflammatory or antipathogenic effects were increased in abundance, and these may be beneficial to HC. We also observed that five of the nine fungal genera that were enriched in UVT patients were opportunistic pathogens. This is the first study demonstrating dysbiosis in the gut fungal microbiomes of UVT patients compared to HC.

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